308 HEPATIC IRON DISTRIBUTION IN 153 PATIENTS WITH CHRONIC VIRAL HEPATITIS (CH): RELATION WITH GRADING, STAGING AND HFE MUTATIONS

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Background: Hepatic iron overload is common in patients with CH. The relationship between iron, grading and staging and the role of HFE mutations in the developement of iron overload and fibrosis are controversial.
Aim: To evaluate the relationship between a) hepatocellular iron distribution, grading and staging; b) HFE genotypes, hepatocellular iron distribution and fibrosis.
Methods: 253 CH patients that underwent liver biopsy from 1995 to 1999 were studied. Hepatic iron was assessed by Deugnier's score modified and grading and staging by Ishak's score. HFE mutations were assesed in 147 patients and in 139 healthy controls.
Results: Hepatic iron overload was present in 124 patients (49%) (group A) and absent in 129 (51%) (group B). Two groups did not differ for age, sex and body mass index (BMI). Hepatic iron store did not correlate with BMI. Fibrosis and steatosis grade were significantly higher in group A than B ($p < 0.0001$ and $p = 0.05$). Cirrhosis was more frequent in group A than B (27% vs 13%, $p = 0.007$). In group A the staging score was significantly correlated with portal iron ($p < 0.001$), particulary with connective and endothelial deposits ($p < 0.001$ and $p < 0.01$). C282Y and H63D were more frequent in group A than in B ($p = 0.033$) and in controls ($p = 0.0002$). A significant correlation between HFE genotypes and hepatocytic iron, that increases with the increasing HFE genotype severity, was found ($p = 0.0014$).
Conclusion: The correlation between fibrosis and portal iron support the fibrogenetic role of macrophagic iron. HFE mutations contribute to the pathogenesis of hepatocytic iron accumulation in CH.

309 LAMIVUDINE-RESISTANT HBV IS CROSS-RESISTANT TO L-dT AND L-dC IN VITRO

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Objective: Clinical resistance of HBV to lamivudine is hallmarked by the development of rtM204I or rtL180M + rtM204V mutations in the viral polymerase. These mutations confer in vitro resistance to lamivudine (beta-L-2'-3'-dideoxy-3'-thiacytidine) and structurally-related L-nucleoside analogs such as FTC and L-FMAU. Novel L-nucleosides with anti-HBV activity have recently been reported; these include beta-L-thymidine (L-dT) and beta-L-deoxycytosine (L-dC), which are each in phase I/II clinical trials. The aim of this study, was to determine whether lamivudine-resistant HBV was sensitive to L-dC and L-dT in vitro.
Methods: HepG2 cells were transfected with wild-type, rtL180M, rtM204I, or rtL180M + rtM204V mutant HBV and were treated with L-dC or L-dT. Following drug treatment, intracellular replicative intermediates were quantified by Southern blotting and the IC50 of each drug was calculated for each strain of HBV.
Results: L-dC and L-dT inhibited the replication of wild-type HBV with IC50 values of 0.26 and 0.28 microMolar, respectively. The rtL180M mutation conferred approximately 10-fold resistance to both compounds, similar to the level of resistance conferred to 3TC. The rtM204I and rtL180M + rtM204V mutations conferred high levels resistance to both L-dC and L-dT (IC50 values increased >300-fold).
Conclusions: These in vitro studies indicate that HBV mutants selected during lamivudine therapy are cross-resistant to L-dC and L-dT and provide further evidence that YMDD mutations confer broad cross-resistance to L-nucleosides.

310 ULTRASTRUCTURAL HEPATOCYTE MODIFICATIONS IN HCV INFECTED HUMAN LIVER

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Despite many efforts, the search for the morphological features of HCV inside the hepatocytes has been hampered by the low level of viral particles present in the liver tissue of infected individuals. Little information is avalaible on intracellular localization of the virus and on prerequisites for its assembly, as a consequence poorly understood is the pathogenic mechanism of liver disease. In this work we analysed, by transmission electron microscopy, 30 hepatic biopsies from HCV infected patients. Liver samples were obtained from individuals infected by hepatitis C virus with different genotypes, and with a different degree of liver damage in order to find possible common ultrastructural modification useful to understand the progression of the disease. A variety of alterations were frequentely seen in hepatocytes, mostly concerning alteration of mitochondria, dilatation of endoplasmic reticulum, and presence of lipid inclusions in the cytoplasm. In addition, in a limited number of hepatocytes we have found peculiar changes of nuclear morphology (indentation and chromatin condensation). The most relevant result is that we found, in association with nucleus modification, the presence of virus-like particles. The morphology and the size of the particles were consistent with the predicted HCV virions based on other studies.

311 DIFFERENT MODULATION OF PKR GENE EXPRESSION BY HCV GENOTYPES

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In hepatitis C, the cellular protein kinase PKR may play a role in the pathogenesis and response to interferon. Some HCV proteins, including NS5A, were shown in vitro to bind PKR and to upregulate or downregulate its expression and activation. Little is know on the in vivo expression of PRK in patients with hepatitis C. Our aim was to measure PKR expression in HCV positive patients. PKR-mRNA was measured by competitive RT-PCR in PBMCs from 24 patients (18 with HCV-1 and 6 with HCV-2) and from control healthy subjects. PBMCs were studied before and after incubation with IFN. Mean baseline PKR mRNA levels were significantly lower in HCV1 patients (0.40 + 0.24 fg) compared to HCV2 cases (2.45 + 2.9 fg $p = 0.006$) and controls (1.37 + 1.0 $p = 0.005$). No correlation was found between mutations in the NS5A-PKR binding domain and PKR-mRNA levels, that were also independent of viraemia, ALT and histologic activity. After in vitro incubation with IFN, PKR mRNA was enhanced to similar levels in HCV1 (3.3 + 2.3) and HCV2 (3.2 + 2.4) patients but remained significantly lower compared to control (8.0 + 5.6 $p = 0.003$). These results indicate that PKR gene expression is downregulated in HCV infection, particularly in patients with HCV1, and this is independent of the NS5A PKR-binding domain sequence. Preliminary evidence suggests that modulation of PKR may affect response to IFN therapy, currently ongoing in these patients.

312 HBV GENOTYPING ANALYSIS FROM THE SURAFCE ANTIGEN REGION USING THE TRUGENE™ HBV GENOTYPING KIT AND A PCR-BASED HOMEBREW METHOD

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Background: Hepatitis B virus (HBV) infects approximately 400 million people worldwide, of those 5–10% become chronically infected. Recent reports suggest some HBV genotypes may influence the outcome of chronic